Ultimately, distinguishing between ecological and sexual causes of sexual dimorphism is not always straightforward because sexual selection is often closely tied to ecology [12] and sexually selected traits are equally associated with ecologically-mediated viability costs. In turn, sex differences in ecology can often be attributed to sexual selection favouring sexually divergent use of resources. Although the distinction is valuable for operational purposes, the line between the two causes is not only blurry, it is somewhat beside the point. Irrespective of the selective mechanisms (be they 'ecological' or 'sexual'), sexual dimorphism reflects the operation of multiple selective factors that, combined, exert sexually antagonistic *net* selection. The most elusive goal for all who study phenotypic evolution, including the phenomenon of sexual dimorphism, is inferring the selective mechanisms that have shaped this pattern of variation. Cooper's study [8] nicely illustrates one way to do so, applying a classic approach of analyzing variation in morph frequencies to the problem of sex-specific polymorphism.

Undoubtedly, the sexes are often subject to very different selection, and in light of this, one might wonder why sexual dimorphism is not even more frequent. One possibility is that a common genetic architecture underlying sexually homologous traits limits the independent evolution of the sexes [13]. However, these intersex genetic correlations are expected to break down over time and some open questions in this field are concerned with the degree to which these correlations might constrain the evolution of dimorphism and/or adaptation [14,15]. Sexual dimorphism almost inevitably reflects past sexually antagonistic selection, but whether it reflects resolved (intralocus) sexual conflict is debatable and has only recently begun to be investigated in any detail [16,17].

We have learned a lot since Darwin and Wallace famously disagreed about the primacy of selective mechanism generating sexual dimorphism, but in some ways old debates continually bubble away beneath the surface, only to rear up and reignite from time to time. We should certainly be aware that sexual dimorphism is not a carte blanche indicator of sexual selection, and equally sexual dimorphism may not mean sexual conflicts are resolved. In short, there is still a lot to learn and it seems the long-standing interest in sexual differences is likely to continue for some time yet.

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Vascular Lumen Formation: Negativity Will Tear Us Apart

Functional blood vessels are essential for vertebrate development, but how endothelial cells initiate lumen formation during vasculogenesis is not known. A new study now reveals that electrostatic repulsion is key.

Renée M. Robbins and Greg J. Beitel*

Establishing patent blood vessels is an essential milestone for developing vertebrates, but exactly how cord-like clusters of endothelial cells create a lumen during vasculogenesis has not been established. Several sialomucins, including CD34 and the podocalyxin-like protein PODXL, localize to presumptive lumenal faces, which suggests that they might assist in lumen formation [1–3]. Sialomucins are transmembrane proteins that are extensively glycosylated and modified with sialic acid on their extracellular domains [3]. Although there are multiple possible functions for sialomucins in lumen formation [3,4], an appealing and almost 30-year-old hypothesis is that the negatively charged sialic acid creates electrostatic repulsion that helps separate the lumenal faces [5–7]. In a study published in this issue of *Current Biology*, Strilić *et al.* [8] now provide strong support for this hypothesis using an impressive combination of *in vivo* and *in vitro* approaches, ranging from pharmacological treatments of embryos to atomic force microscopy to a clever new cell adhesion assay.

This group previously showed that, during vasculogenesis, the mouse

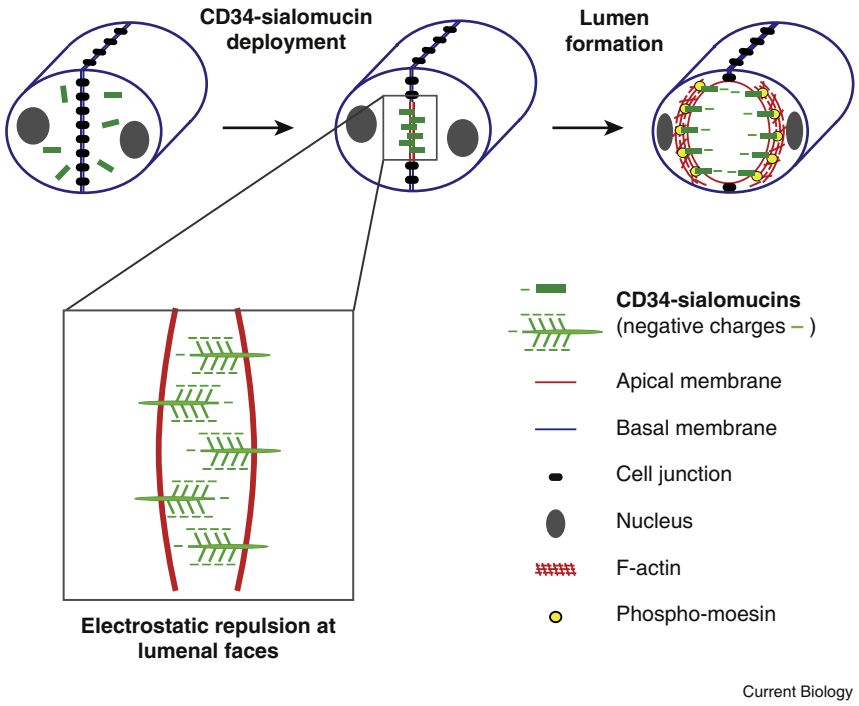

Figure 1. Initiation of vascular lumen formation by electrostatic repulsion.

During vasculogenesis, the lumen in a developing murine dorsal aorta forms by 'cord hollowing' [1]. A long-standing, but unproven, hypothesis proposes that electrostatic repulsion by negatively-charged apical glycoproteins initiates lumen formation by separating apposing cell surfaces [5–7]. In this issue, Strilić *et al.* [8] now provide strong *in vivo* and *in vitro* support for this model. (Adapted from [1,9].)

dorsal aorta forms a lumen by 'cord hollowing', a mechanism in which cells in a cylindrical cord of endothelial cells polarize, reorganize their junctions, and then open a central lumen inside the cord (Figure 1) [1,9,10]. In this process, apical glycoproteins, including CD34 and PODXL, are delivered to the cell–cell contacts where the lumen will form. Then the incipient lumenal faces of the endothelial cells de-adhere from each other and form the lumen [1]. Thus, the sialomucins are in the right place at the right time to initiate lumen formation. But does their electrostatic charge actually drive lumen formation?

It is not obvious that the answer to this question is 'yes', since mice lacking CD34 or PODXL do form aortae [11,12]. Furthermore, apical glycoproteins, including PODXL, have non-electrostatic functions, such as scaffolding via their intracellular domains [3,4]. In particular, during murine aortal development, the intracellular domain of PODXL binds to a phosphorylated form of moesin, which in turn organizes apical actin in endothelial cells [1,13]. In the absence of either PODXL or moesin phosphorylation, moesin is not efficiently recruited to the apical surface and lumen formation is delayed [1]. Thus, although PODXL is required for normal lumen formation, the phenotype of PODXL-deficient animals does not establish a role for electrostatics in the process. Similarly, the phenotype of *Caenorhabditis elegans* kidney tubules lacking the secreted mucin Let-653 suggests that Let-653 contributes something other than electrostatic repulsion to lumen morphogenesis. Electrostatics would predict that loss of *let-653* should reduce or prevent lumen formation, whereas in fact *let-653* mutants have an expanded lumen [14]. Thus, despite the 'attractiveness' of electrostatic repulsion as a mechanism to initiate lumen formation, experimental support for this hypothesis has been lacking.

Undaunted by the paucity of encouraging evidence, Strilić *et al.* [8] set out to show that electrostatic repulsion is an essential role of sialomucins in vascular lumen formation. First, they tested whether the highly charged extracellular sialic acid modifications were in fact necessary for lumen formation by enzymatically removing them using neuraminidase, and also by neutralizing the negative charge of sialic acid with cationic protamine sulfate. Both treatments greatly reduced lumen formation in early mouse embryos undergoing vasculogenesis, and in an *in vitro* angiogenic sprouting assay. Critically, neither treatment altered *in vivo* events that precede lumen formation, including the localization of PODXL, moesin, and F-actin to the apical cell surface. These results provide strong evidence that the electrostatic charges of sialomucins are important for lumen formation. However, the findings did not distinguish whether the role of the sialic acid was to provide a general negative charge that created repulsion, or to provide a feature that mediates a specific binding interaction. Remarkably, Strilić *et al.* [8] were able to restore both *in vivo* and *in vitro* lumen formation after neuraminidase treatment with a subsequent treatment using dextran sulfate, which binds cell surfaces and is negatively charged, but has a different chemistry from that of sialic acid. Conversely, subsequent treatment with uncharged dextran did not restore lumen formation *in vivo* or *in vitro*. These results demonstrate that the negative charges on proteoglycans are critical for opening a lumenal space.

Having established that electrostatics are necessary for lumen formation, Strilić *et al.* [8] turned to testing whether eliminating the negative charge on endothelial cell sialomucins increased endothelial cell adhesion, as predicted by the hypothesis that electrostatic repulsion helped separate apposing cell faces to create a lumen. To test cell–cell adhesion, they developed a novel assay that should have wide applicability, as it is elegant in its simplicity and much more accessible than techniques such as atomic force microscopy. In this 'bead rolling assay', human umbilical vein endothelial cells (HUVECs) that express sialic acids at their apical surface are grown both on beads and on cell culture dishes. The cell-coated beads are then placed onto the monolayer of cells in the dish, which is then tilted. The distance that the bead rolls inversely correlates with

the adhesive strength of the cells. When the cells were treated with neuraminidase or protamine sulfate, the distance the bead traveled was greatly reduced. Conversely, when negatively-charged dextran sulfate, but not uncharged dextran, was added to the neuraminidase-treated cells, the distance the bead traveled was significantly restored. These results were confirmed using single-cell force spectroscopy (SCFS), a variation of atomic force microscopy. Together, the work of Strilić *et al.* [8] shows that negative charge strongly decreases the adhesion between endothelial cell surfaces and facilitates lumen formation. Thus, the long-standing hypothesis that electrostatic repulsion can drive lumen formation now has strong experimental support.

Does electrostatic repulsion play a role in lumen formation in non-endothelial tubes? Many epithelial tubular organs, including the kidney and lung, express mucins and glycoproteins on their lumenal surfaces [14–17]. Cell–cell repulsion has been observed in the formation of the *Drosophila* heart, which arises from mesodermal cells that converge to encapsulate a lumen [18–20]. Thus, electrostatics could be critical for lumen morphogenesis in many organs, and Strilić *et al.* [8] have provided experimental approaches for determining whether charge is a key for opening the lumenal space.

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Numerical Processing: Stimulating Numbers

A new study using transcranial direct current stimulation shows that modulating parietal cortex activity during the learning of abstract numerical material can enhance numerical competency for up to six months.

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Numerical competency is a fundamental ability that is present across many species [1]. In humans, numbers are so important that impairments in mastering the manipulation of these abstract concepts and symbols — a condition named dyscalculia — can lead to serious personal, social, and economical difficulties. For example, poor numerical skills have been associated with erroneous patient dosing of medication [2]. Developmental dyscalculia is characterized by a marked inability to perform even simple numerical operations [3]. Affecting approximatively 5% of the population [4], developmental dyscalculia cannot be explained by low intelligence, sensory difficulties or poor schooling [5]. Although recent advances in cognitive remediation seem promising [6], results so far suggest that the positive effects associated with the use of computer-assisted interventions, for example, are short-lived [7].

Neuroimaging studies looking at the neural basis of numerical ability have shown that the brain network recruited for mathematical processing depends largely on the type of material used and the specificity of the task [5]. Nonetheless, a consistent finding in these studies is the involvement of the posterior parietal cortex, more specifically the horizontal segment of the intra-parietal sulcus (IPS) [8]. Functional magnetic resonance